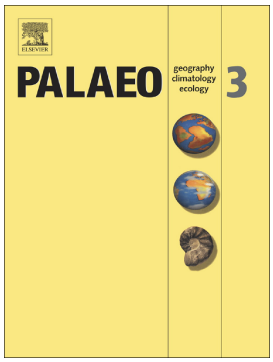

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Implications of the apparent ^{14}C age of cultured *Achatina fulica* and the spatial features of ^{14}C ages among modern land snail shells in China

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ABSTRACT

It has long been known that many terrestrial snail shells are subject to the so-called "limestone effect". However, the magnitude and impact of this effect on the $\delta^{13}C$ values of snail shells and soft body tissue ($\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$) are still unknown. In this study, *Achatina fulica* snails were hatched and cultured under controlled conditions and supplied with ^{14}C-free calcium carbonate and lettuce (a C_3 plant) as a calcium source and food supply, respectively. The snail shells and soft body tissues were sampled monthly for 5 months, and $\delta^{13}C$ and radiocarbon dating analyses were conducted to trace carbon source variations at different growth stages. Our results show that the apparent ^{14}C age of the shells and the corresponding $\delta^{13}C_{shell}$ values increased progressively from the 1st to 3rd months and subsequently decreased during the 4th and 5th months. The results demonstrate for the first time that carbonate ingestion has a straightforward influence on the ^{14}C ages and $\delta^{13}C$ values of shells and that the "limestone effect" changes over the lifetime of snails. Moreover, the apparent ^{14}C ages of snails sampled in the field are older than modern ages when taken from the northern carbonate region and relatively young or the same as modern ages when collected from ferralic soils. This further supports our findings from cultured snails and suggests that snail shells are good archives for radiocarbon dating purposes in southern China.

Although the $\delta^{13}C$ values of the soft body tissues of cultured snails vary synchronously with those of the shell, this seemingly common phenomenon cannot be easily explained by carbonate ingestion, as the snail bodies are of more recent origin

than the shells. Atmospheric CO_2 may be another candidate explaining the synchronicity between $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$, as well as the F ^{14}C variations of snail bodies. However, it is still unclear how snails differentially incorporate carbon from different sources (e.g., carbonate and atmospheric CO_2) at different growth stages, since carbon from different sources is combined in the bicarbonate pool in the hemolymph before being used by snails.

Key words: Limestone effect, AMS dating, Radiocarbon age

1. Introduction

Land snail shells are widely distributed and well preserved in terrestrial sediments, especially in loess-paleosol sequences in China (Liu, 1985; Rousseau and Wu, 1997; Wu et al., 2018), and are often available for dating in paleoenvironmental, geological and archaeological studies. Because dietary calcium is an essential nutrient for growth and reproduction and shell construction in gastropods (Crowell 1973, Tompa and Wilbur 1977), terrestrial snails often consume limestone and other aged carbonates as a calcium source where they are available (Goodfriend and Stipp, 1983). Therefore, the use of aragonite snail shells for ^{14}C dating is restricted by the well-known limestone problem, which refers to the incorporation of relatively ^{14}C-depleted carbonate (old carbon or dead carbon) into shells (Goodfriend and Stipp, 1983). Studies have shown that the amount of old carbon can be highly variable, ranging from negligible to ~30%, which could cause age anomalies of up to ~3000 ^{14}C years too old (Rubin and Berry, 1963; Tamers, 1970; Evin et al., 1980; Goodfriend and Stipp, 1983; Goodfriend et al., 1999; Yates et al., 2002; Quarta et al., 2007; Romaniello et al., 2008; Xu et al., 2010, 2011; Pigati et al., 2004, 2010, 2013, 2015).

Three approaches are often used to study the limestone effect on snail shell ^{14}C ages. One approach is to measure modern shells of different species from various natural environments (Tamers, 1970; Evin et al., 1980; Goodfriend and Stipp, 1983; Goodfriend et al., 1999; Yates et al., 2002; Quarta et al., 2007; Romaniello et al., 2008; Xu et al., 2010, 2011; Pigati et al., 2004, 2010). An alternative approach is to compare

the fossil shell ^{14}C ages with independent ages constrained by their time of deposition, such as the ^{14}C ages of charcoal or plant macrofossils (Brennan and Quade, 1997; Pigati et al., 2013). A third approach is to raise snails under controlled conditions to evaluate the factors that influence ^{14}C uptake (Rubin and Berry, 1963). Many studies have focused on the first two approaches, while few studies chosen to use the time-consuming approach of raising snails to study the limestone problem. Moreover, the ^{14}C variability during the lifetime of a snail is still unclear and could shed light on the reliability of the use of snail shells for radiocarbon dating and provide useful information on the selection of suitable shells for ^{14}C dating.

Currently, the models for snail shell formation suggest that both carbon from carbonate and atmospheric CO_2 can be incorporated into the hemolymph of a living snail and then incorporated into its shell (Goodfriend and Hood, 1983; Zhang et al., 2014). Stott's model indicates that the CO_2 dissolved in the hemolymph is partly transferred into the snail shell and partly synthesized into arginine by arginase (Stott 2002). This implies that the old carbon from carbonate may be transferred into body tissues and that the $\delta^{13}C$ of soft tissues is influenced by both carbonate and atmospheric CO_2. However, the soft tissues of snails have been shown to have modern^{14}C ages on the Chinese Loess Plateau (Xu et al., 2010, 2011), and the $\delta^{13}C_{body}$ values primarily reflect the carbon isotopes inherited from the snails' diet in laboratory experiments (Deniro and Epstein, 1978; Stott, 2002; Metref et al., 2003; Zhang et al., 2014). Thus, strong evidence is needed to evaluate the proportional amounts of different carbon sources in the body tissues of snails and the mechanism

of selective carbon uptake in snails if the models are correct.

Over the last several decades, stable isotope signatures of the snail shell have been widely used for environmental and palaeoclimatic studies (Yapp, 1979; Magaritz et al., 1981; Magaritz and Heller, 1980, 1983; Lécolle 1985; Goodfriend 1988, 1992, 1999; Bonadonna and Leone, 1995; Leng et al., 1998; Goodfriend and Ellis, 2000; Leone et al., 2000, 2002; Zanchetta et al., 2005, 2017; Balakrishnan et al., 2005a; Colonese et al., 2007, 2014; Li et al., 2007; Liu et al., 2007; Yanes et al., 2008, 2009, 2011, 2012, 2013, 2014, 2017, 2018a,b; Kehrwald et al., 2010; Huang et al., 2012; Yanes 2015; Prendergast et al., 2015, 2016, 2017; Wang et al., 2016; Bao et al., 2018). $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$ are generally regarded as a reflection of the carbon sources in a snail's diet and are indicative of the vegetation type ingested (Goodfriend and Magaritz, 1987; Goodfriend1992; Goodfriend and Ellis, 2002; Balakrishnan and Yapp, 2004; Balakrishnan et al., 2005b; Baldini et al., 2007; Liu et al 2007; Prendergast et al., 2017; Yates et al., 2018). These results are consistent with laboratory snail-feeding experiments (Deniro and Epstein, 1978; Stott, 2002; Metref et al., 2003; Zhang et al., 2014). However, some studies also suggest that $\delta^{13}C_{shell}$ may be affected by inorganic carbon uptake, such as from a carbonate substrate or from carbonate minerals in soil sediments (Goodfriend and Stipp, 1983; Yates et al., 2002; Yanes et al., 2008; Zhang et al., 2014). For example, Goodfriend and Hood (1983) observed that the $\delta^{13}C_{shell}$ value increased as the contribution of limestone increased. Yanes et al. (2012) reported that higher $\delta^{13}C_{shell}$ values were observed during the juvenile stages of both living and fossil snails, indicating a higher contribution of ingested limestone. This

discrepancy may be attributed to (1) the lack of direct evidence related to the contribution of digested carbonate to the $\delta^{13}C_{shell}$ value, (2) the use of a single proxy to trace carbon sources, such as $\delta^{13}C$, or (3) the neglect of the snail living environment.

Thus, the primary goals of this study were to culture snails under controlled conditions to 1) study the ^{14}C age anomalies during different life periods of land snails and assist the selection of appropriate shells for AMS dating purposes and 2) quantify the contributions of different carbon sources in snail shells and soft body tissues based on the $\delta^{13}C_{shell}/\delta^{13}C_{body}$ and ^{14}C dating results. To confirm our culturing experiment, we also conducted a regional comparison of ^{14}C data derived from snail shells collected from carbonate-poor areas in southern China to carbonate-rich soils in northern China, as well as previously published results from Xu et al. (2010, 2011).

2. Materials and methods

The *Achatina fulica* "white" land snail, also called the “White Jade” snail in China because of its jade-like white body, is an albino morph of the normal Giant African snail *Achatina fulica*. Its lifespan, which can be up to 10 years, is usually 5-7 years, and its sexually maturity can be reached as early as 5 months of age. Furthermore, the average adult shell length is ~12 cm. Due to these features, this species was chosen for the culturing experiment. White Jade snail eggs were hatched, and snails were raised on a diet of lettuce (C3-type plant, ~ -27.5 ‰, F ^{14}C value 0.99) under controlled temperatures (~27℃) and humidity (~85%) in the laboratory in an incubator. F ^{14}C was defined by Reimer et al. (2004). Loess deposits from the

penultimate glaciation (>130 thousand years ago, ^{14}C-free according to Peng Cheng's unpublished data, ~10% carbonate content, $\delta^{13}C$=−5.7‰) on the Chinese Loess Plateau were also supplied to the snails as a source of calcium. Here, this material is known as ^{14}C-free, as its age far exceeds the limit of the radiocarbon age dating technique (~50 ka). Cultured snail shells and soft tissues were collected monthly for 5 months, and 5 shells (Figure 1), 5 soft body samples and one snail egg sample were studied.

Fresh modern land snail shells were also collected from the middle part of China to Hainan Island in southern China in this work (Figure 2) to further elucidate the possibility of using land snail shells for radiocarbon dating because previous radiocarbon studies on modern land snails mainly focused on the Chinese Loess Plateau in northern China (Figure 2, Xu et al., 2010, 2011). Here, we focused on two genera, namely, *Bradybaena* and *Achatina*. The former genus is widely distributed in China, and the latter genus is mainly active in regions of southern China, such as Xiamen, Guangzhou and Sanya.

Before ^{14}C dating, the shells, soft tissues and lettuce were treated with 5% H_2O_2 and 10% H_3PO_4 in a beaker overnight to remove adhered organic matter and soil carbonates, respectively. The samples were subsequently rinsed with distilled water and dried. Shells were not powdered during pretreatment to minimize the potential for atmospheric ^{14}C adsorption. The shells were washed repeatedly in 18.2 M (hereafter "ultrapure") water, sonicated for a few minutes to remove adhered material, washed again with ultrapure water, and dried in a vacuum oven overnight at ~70℃. Shell

aragonite was converted to CO_2 using 100% H_3PO_4 under vacuum at 75℃. Water, SO_x, NO_x, and halide species were removed using a trap with a liquid nitrogen and alcohol mixture, and the purified CO_2 was used for ^{14}C analysis. The organic tissue was combusted using CuO powder at 900℃, and the resulting CO_2 was split into 2 aliquots. One aliquot of CO_2 was converted to graphite using an Fe catalytic method (modified after Slota et al. 1987), and ^{14}C was measured at the Xi'an AMS center facility. The primary standard NIST OXII was used for the normalization and evaluation of the overall procedure accuracy and precision, while anthracite was used to estimate the background correction.

Another aliquot of CO_2 for $\delta^{13}C$ was analyzed with a MAT 251 isotope ratio mass spectrometer at the Institute of Earth Environment, CAS, and the national standard material (GBW04407, $\delta^{13}C = -22.61‰$) was analyzed during the experiment. The $\delta^{13}C$ values of the cultured snail shells reported here were reacted with 100% phosphoric acid, and the extracted CO_2 was analyzed on a Finnigan MAT 253 gas source isotope ratio mass spectrometer at Caltech, Pasadena, CA. The laboratory standards of TV04 ($\delta^{13}C = 1.47$ ‰) and CIT Carrara ($\delta^{13}C = 2.11$ ‰) were measured along with the samples.

A two-endmember mass balance equation was used to determine the proportions of old and modern carbon in the snail shell, assuming an F ^{14}C value of 1, which is almost identical to the F ^{14}C value of the lettuce, for the modern carbon endmember and an F ^{14}C value of 0.0019 for the carbonate endmember (Peng Cheng's unpublished data).

$F_{shell} = 0.0019 \times X + 1 \times Y$ and $X + Y = 1$, then, $X = 100 \times (1 - F_{shell}) / 0.9981$ (1)

where F_{shell} is the measured F ^{14}C value of the snail shells, X is the proportion of old carbon from carbonate, and Y is the proportion of modern carbon (including carbon from the snail diet and from atmospheric CO_2). Thereafter, Equation 6 and the related parameters in Xu et al. (2010) were adopted to further calculate the proportions of carbon from the snail diet and from atmospheric CO_2. The results are shown in Table 1.

3. Results

Table 1 lists the shell weight, AMS dating and stable carbon isotope results, as well as the calculated proportions of carbon from different sources, for the cultured snails. Figure 1 shows an aperture view of different aged shells of *Achatina fulica*. Obviously, the shells became larger, thicker and more colorful with time, and the length of the shell grew from ca. 1 cm in the 1st month to more than 7 cm in the 5th month. Meanwhile, the shell weight increased from 0.12 g to 8.99 g. The shell weight increased rapidly from 0.12 g in the 1st month to 4.61 g in the 3rd month and then increased more slowly in the 4th and 5th months from 8.53 g to 8.99 g, although the absolute weight gain of the shell was still increasing (Figure 3, Table 1).

The F ^{14}C value of the shells varied between 0.8313 and 0.7304, corresponding to apparent ^{14}C ages of ~2520 yr. to ~1480 yr., but the snail soft tissue F ^{14}C values were relatively constant, with a value of approximately 1, suggesting a modern carbon origin (Table 1). The snail egg showed an apparent ^{14}C age of 740 yr. As the shell

weight increased, the shell F ^{14}C values decreased from the 1st to 3rd month and then increased with the shell weight in the 4th and 5th months (Figure 4, Table 1). The $\delta^{13}C$ values of the snail shells and soft tissues varied from -11.31 to -14.23‰ and from -27.68 to -24.81‰, respectively (Figure 5, Table 1), and the difference between the shells and soft tissues was relatively constant, with an average value of 13‰. Interestingly, $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$ showed synchronous variations with the F ^{14}C value of the shells, and both $\delta^{13}C$ values became more positive from the 1st to 3rd month (Figure 4, Figure 5). The most positive $\delta^{13}C$ value occurred in the 3rd month, with the smallest F ^{14}C value and the largest proportion of carbonate (Table 1). This clearly shows the direct influence of carbonate ingestion on the shell $\delta^{13}C$ and F ^{14}C values.

The F ^{14}C values varied between 0.8939 and 1.0208 for modern snail shells collected from the middle part of China to southern China (Figure 2, Table 2). These values corresponded to a range in apparent ^{14}C ages from 901 yr. to modern times (Table 2). This offset decreased from north to south in China. The ferralic soil in southern China is poor in calcium carbonate because the soluble salt and carbonate have been leached out due to strong weathering. Thus, the ^{14}C ages of the snail shells were equivalent to or close to the modern age in southern China, with minimal limestone effects. This feature is discussed further below.

4. **Discussion**

The carbon in land snail shells has three potential sources, food, atmospheric CO_2, and ingested carbonates (Goodfriend and Hood, 1983), whose relative contributions

remain unclear. Here, we discuss the possible influences of these carbon sources in snail shells and soft tissues and assess the reliability of snail shells for ^{14}C dating based on our AMS ^{14}C dating and stable carbon isotope results.

4.1 The influence of carbonate ingestion on snail shells

One of the most prominent features of our data is the covariation in the $\delta^{13}C_{shell}$ and F ^{14}C values and the positive shifts in $\delta^{13}C_{shell}$ (from -14.23‰ to -11.31 ‰) and corresponding decrease in the F ^{14}C values in the 3rd month (Figure 4). It seems that the fluctuation in $\delta^{13}C_{shell}$ may be simply explained by changes in the carbon isotopes of food because food is regarded as the dominant control on the isotopic compositions of snail shells and soft tissues (DeNiro and Epstein 1978; Stott 2002; Metref et al 2003; Liu et al., 2007; Zhang et al., 2014). However, it is unlikely that the amplitude of $\delta^{13}C_{shell}$ (~3‰) can be explained by variations in the stable carbon isotope of the food because we only used lettuce (C3 type plant, ~-27.5‰) as a dietary carbon source in the experiment. Furthermore, the dietary carbon is of modern origin, and it could not induce such large changes in the F ^{14}C. Thus, this phenomenon cannot be fully explained by changes in the dietary isotopic composition.

According to the models for shell formation (Goodfriend and Hood, 1983; Zhang et al., 2014), positive $\delta^{13}C_{shell}$ shifts may indicate increased contributions from atmospheric CO_2 because the $\delta^{13}C$ of modern atmospheric CO_2 (-8‰, Rubino et al., 2013) is more positive than that of lettuce (~ -27.5‰). In this perspective, however, the carbonates ($\delta^{13}C$= ~ -5.7‰) we used could produce an effect similar to that of atmospheric CO_2 because the carbon from both sources can be introduced into the

bicarbonate pool in the snail hemolymph, from which shell carbonate is ultimately precipitated (Figure 6, Goodfriend and Hood, 1983; Zhang et al., 2014). Although we cannot distinguish the effects of these sources on $\delta^{13}C_{shell}$ based on their stable carbon isotopes, the ^{14}C contents of the two sources are quite different and can be used to distinguish between them. Atmospheric CO_2 has a similar amount of ^{14}C as snail food. Therefore, atmospheric CO_2 may only influence the $\delta^{13}C_{shell}$ value and does not strongly affect the shell's F ^{14}C value.

Our calculation suggests that atmospheric CO_2 and carbonates make similar contributions (10%~30%) to $\delta^{13}C_{shell}$ at different growth stages, and both contributions peak at the 3rd month (Table 1). These results are consistent with previous work by Xu et al. (2010, 2011) and Zhang et al. (2014) but inconsistent with other studies indicating negligible (Stott, 2002; Metref, 2003) or substantial (Goodfriend and Hood, 1983; Romaniello et al., 2008) contributions from atmospheric CO_2. This demonstrates the complexity in determining the role of atmospheric CO_2 in the $\delta^{13}C_{shell}$ value.

The changes in our snail shells' F ^{14}C values further confirm the effect of limestone and its impact on the $\delta^{13}C_{shell}$ values in terrestrial snails, who consume carbonates when building their shells (Goodfriend and Stipp 1983). The F ^{14}C variations could be well explained by the ingestion of different amounts of carbonate according to previous studies (Goodfriend and Hood 1983; Pigati et al., 2004; Quarta et al., 2007; Romaniello et al., 2008; Xu et al., 2010, 2011). Considering the ^{14}C age of shells collected in the field from northern to southern China (Table 2, Xu et al.,

2010, 2011), the relatively positive $\delta^{13}C_{shell}$ values for the northern snail species and negative $\delta^{13}C_{shell}$ values for the southern snail species may also be related to the intake of carbonates (Bao et al., 2018); thus, $\delta^{13}C_{shell}$ may be a more reliable indicator of local vegetation in southern China than in northern China due to the influences of soil carbonate. Moreover, the mean offsets of $\delta^{13}C_{shell}$ from $\delta^{13}C_{body}$ from carbonate soil regions in northern China (14.3‰, Liu et al., 2007) are larger than those from southern China and from Stott's carbon isotope fractionation model (12.7‰) (Bao et al., 2018; Stott 2002). Therefore, the covariation in the $\delta^{13}C_{shell}$ and F values in Figure 4 strongly suggests ingested carbonates have a direct influence on F ^{14}C and stable carbon isotope values.

Furthermore, the limestone effect may be the byproduct of only calcium intake from the environment. Dietary calcium affects the growth of terrestrial snail shells (Egonmwan 2008; Hotopp 2002; Skeldon et al 2007). Hotopp's study showed that the calcium levels in upper soil horizons are positively correlated with land snail density and species richness (Hotopp, 2002), and the work of Skeldon et al. indicated that snail abundances are probably controlled by calcium levels (Skeldon et al., 2007). It has also been observed that $CaCO_3$-fed snails are relatively heavier than snails fed lettuce alone (Crowell, 1973). We also note that the shells of snails raised with only lettuce were thin, with little change in size, implying that a lettuce-only diet is insufficient to support the development of healthy shells. Hence, calcium absorption from soil carbonate, as observed in this study, or from other sources in the field (such as snails from southern China), may be the ultimate control of shell formation and the

limestone effect.

As discussed above, the most enriched $\delta^{13}C_{shell}$ and lowest F ^{14}C values occurred in the 3rd month, and we interpret this result as an indication that the largest proportion of calcium carbonate ingestion occurred in the 3rd month, suggesting that snails require a relatively large amount of calcium at this stage. This is similar to the study of Egonmwan 2008. Egonmwan's long-term culture experiment also showed that calcium ingestion by snails peaked in the 3rd month (Figure 4 in Egonmwan 2008). Hence, it appears that a relatively large amount of calcium is required for juvenile snails (1st-3rd month). The increased F ^{14}C value and the negative shift in $\delta^{13}C_{shell}$ in the 4th and 5th month may be explained by an increased proportion of plant-derived carbon and/or a decreased proportion of carbon derived from the intake of soil carbonate (e.g., the reduced intake of calcium shown in Figure 4 in Egonmwan 2008). Increased plant consumption could dilute the ingestion of old carbon and result in higher F ^{14}C values and lower $\delta^{13}C_{shell}$ values. This implication also agrees with the positive correlation between leaf litter consumption and shell size (Astor et al., 2015), suggesting an increased consumption of plants by large snails. Moreover, the minimum apparent ^{14}C age of the snail egg further implies that the limestone effect is minimized in adult snails (Table 1). If the increases in the calcium levels in the snail hemolymph during the egg laying period are considered (Tompa and Wilbur, 1977), the age anomaly of snail eggs may be even smaller. As the absolute weight gain of shells was still increasing in the 4th and 5th months (Figure 3, Table 1), the ingested carbonate probably did not decrease, at least in the 4th month in our study. For

example, the increase in shell weight in the 4th month (from 4.61 g to 8.53 g) is similar to the total increment over the first 3 months. Overall, our results reflect the direct influence of carbonate ingestion on the F ^{14}C and $\delta^{13}C_{shell}$ values of snails.

4.2 The influence of atmospheric CO_2 on snail soft tissues

Another important feature of our results is the relatively constant δ^{13}C offset between aragonite shells and soft tissues, i.e., the covariation between $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$ (~13‰, Figure 5). A similar phenomenon has been reported in previous studies (Goodfriend and Ellis, 2002; Liu et al., 2007; Xu et al., 2010; Prendergast et al., 2017; Bao et al., 2018). This constant δ^{13}C offset is fundamental when snail shells are used to reconstruct their food sources because $\delta^{13}C_{body}$ is usually regarded to reflect their diet (DeNiro and Epstein, 1978; Stott, 2002; Metref et al., 2003; Zhang et al., 2014). However, as discussed above, the most positive shift in $\delta^{13}C_{shell}$ is probably related to carbonate ingestion or atmospheric CO_2, not diet. This implies that the carbon isotope signal from ingested inorganic carbonates or atmospheric CO_2 may also be transferred into soft tissue (Figure 5), as carbon from different sources is combined in the hemolymph bicarbonate pool (Figure 6, Goodfriend and Hood, 1983; Zhang et al., 2014), and a portion of the dissolved CO_2 in the hemolymph is synthesized into arginine by arginase (Stott 2002). Furthermore, previous work has demonstrated that snails can incorporate [^{14}C]bicarbonate into arginine in proteins (Campbell and Speeg 1968). This suggests that the contribution of ingested carbonates or atmospheric CO_2 only shifts the carbon isotope value of the snail shell and body to the positive end, while the offsets between $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$ are

unaffected. In this case, care should be taken when $\delta^{13}C_{shell}/\delta^{13}C_{body}$ values are used to reconstruct the types of ingested vegetation or the C3/C4 ratio in the past.

Moreover, the F ^{14}C value of the cultured snail soft tissue was nearly constant, with an F value of approximately 1, implying carbonates made no contribution to the $\delta^{13}C_{body}$ value. Previous radiocarbon dating studies also show that the carbon in the soft tissues of snails is of modern origin (Xu et al 2010, 2011), i.e., the limestone effect has no influence. Indeed, our calculation shows that the contribution of carbonates approaches zero, while it is ~20% for atmospheric CO_2 (Table 1). Therefore, we argue that the positive shift in $\delta^{13}C_{body}$ may be derived from an atmospheric CO_2 source. However, it is still unclear how snails differentially incorporate carbon from different sources (e.g., carbonate and atmospheric CO_2) at different growth stages.

4.3 Reliability of snail shells for ^{14}C dating

The ^{14}C dating results of minute terrestrial snails suggest little or no consumption of ^{14}C-depleted carbonate (Brennan and Quade, 1997; Pigati et al., 2004, 2010), while large snail shells are more susceptible to the limestone effect (Rubin and Berry, 1963; Tamers, 1970; Evin et al., 1980; Goodfriend and Stipp, 1983; Quarta et al., 2007; Romaniello et al., 2008; Xu et al., 2010, 2011). If the limestone effect depends on the size of the snail, the large *Achatina fulica* snails we studied should have a large apparent ^{14}C age, as a relatively high amount of carbonate would be required to build their shells and more old carbon would be consumed during the growing process. Our results indeed show that the age anomaly increased gradually from the 1st month to the 3rd month from ~1645 yr to ~2520 yr, although it decreased in the following two months, probably due to an increased dietary intake of lettuce (Table 1, Figure 4). The age anomaly determined in our study is larger than most previously reported age anomalies (Rubin and Berry, 1963; Tamers, 1970; Evin et al., 1980; Quarta et al., 2007; Romaniello et al., 2008; Xu et al., 2010, 2011). The largest age difference is still ~500 years younger than that reported by Goodfriend and Stipp (1983), and 2520 yr. could represent a maximum age anomaly for *Achatina fulica*.

The results from Goodfriend et al. (1999) are also similar to our findings in some respects. They measured the ^{14}C age of different parts of a single shell and showed larger apparent ages (up to 1200 yr.) in the apical part of the shell, with younger and more uniform ages in the last whorl. As shown in Figure 1, shells become thicker with snail growth, so newly precipitated aragonite was clearly added to the apical part of

the shell as the shell grew. Therefore, we suggest that the body whorl (last whorl) of adult snail shells may be least impacted by the limestone effect and thus could provide a more reliable ^{14}C age than other shell parts, while the apical part of shells should be avoided for AMS dating purposes.

The apparent ^{14}C ages of snail shells are relatively large, ranging from 2400 yrs. to 400 yrs. in carbonate soils in northern China (Table 2, Figure 2, Xu et al., 2010, 2011). However, shells from southern China live on ferralic soils (Table 2, Figure 2), where there are few or no carbonates in the soils due to strong weathering (Bao et al., 2018). This suggests that terrestrial snail shells can provide reliable ^{14}C ages for paleoclimatic or archeological studies in southern China. Moreover, these shells could also be used to reconstruct the atmospheric ^{14}C changes, as reported by Macario et al., 2016, who found that shells were not affected by old carbon in the subtropical region of Brazil, which has similar snail habitats as southern China. This spatial pattern further supports the impact of ingested carbonates on the ^{14}C ages of snail shells observed in our culturing experiment. Therefore, the reliability of snail shells for ^{14}C dating may not depend merely on the size of the snail but also on its habitat and living environment, and reliable ^{14}C dating is favored in soils that are poor in carbonate (Goodfriend and Stipp 1983).

5. Conclusions

We conclude that the "limestone effect" changes over the lifetime of snails. Furthermore, the covariation of the F ^{14}C value and $\delta^{13}C_{shell}$ of a cultured snail demonstrates the direct influence of carbonate ingestion on these parameters, and the

apparent ^{14}C age should increase with the carbonate contribution. The atmospheric CO_2 probably contributes to the variation in both $\delta^{13}C_{shell}$ and $\delta^{13}C_{body}$. However, it is still an open question as to how snails differentially incorporate carbon from different sources at different growth stages.

Our study further shows that most snail shells from ferralic soil habitats, such as those in southern China, do not show a strong limestone effect, while this effect is pronounced in soils rich in carbonate, such as soils in northern China. This spatial pattern of "northern-older" and "southern-younger" ^{14}C ages agrees with our results and supports the limestone effect proposed by (Goodfriend and Stipp 1983). The reliability of the ^{14}C ages of snail shells depends on their habitat and diet, and the most reliable ^{14}C ages can be obtained by sampling the outermost body whorl.

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Figure 1. Aperture view of snail shells collected during the 1st to the 5th month of growth. The figure shows that shells become thicker and more colorful with age. The black line indicates one centimeter.

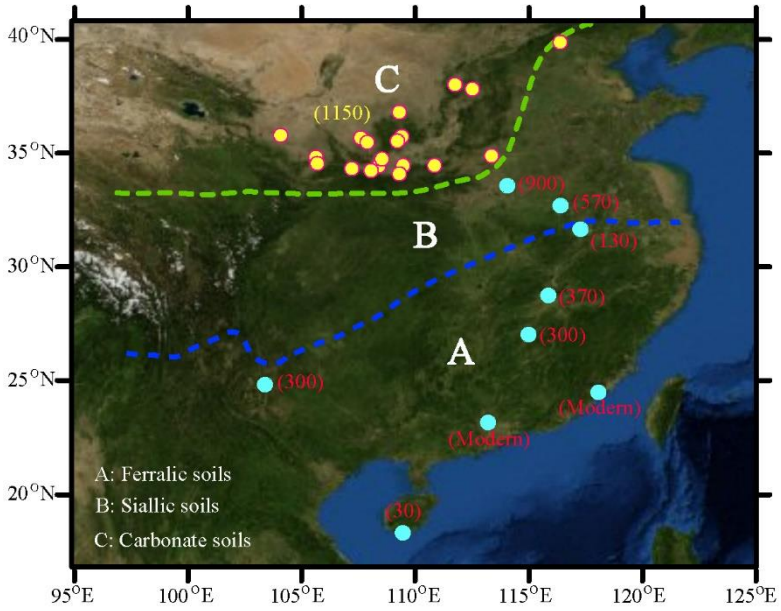

Figure 2. The location of snail shells collected in the field in this study (filled bright green circles) and modern snail shells (filled yellow circles) for AMS ^{14}C analysis reported by Xu et al. (2010, 2011). Dashed lines show the approximate boundaies of the soil types adapted from Gong et al. (1998). The red numbers in brackets show the ^{14}C ages (yr BP) (Table 2), and the yellow numbers in parentheses denote the mean ^{14}C ages for the published results on the Chinese Loess Plateau from Xu et al. (2010, 2011).

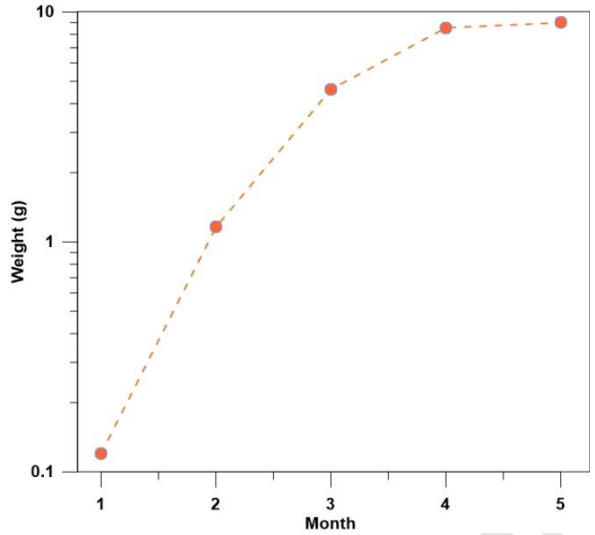

Figure 3. Variations of snail shell weights vs months. The shell weight increases expontentially in the juvenile stage. Note the log vertical scale.

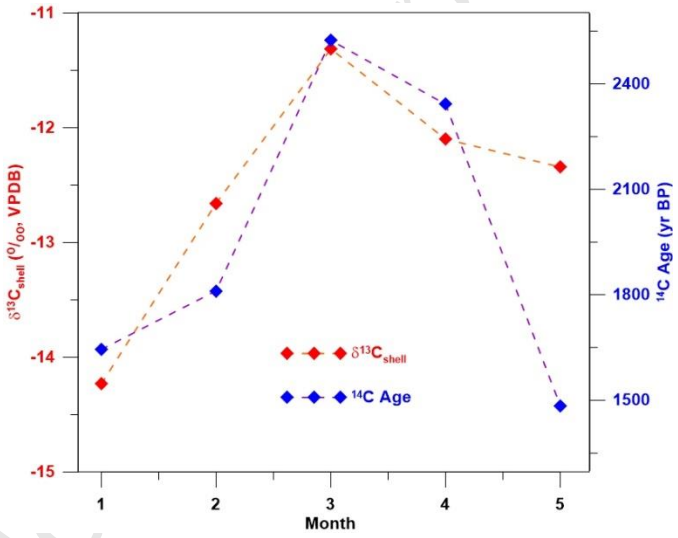

Figure 4. Variations of the snail shell $\delta^{13}C$ (red) and ^{14}C age (blue) vs months

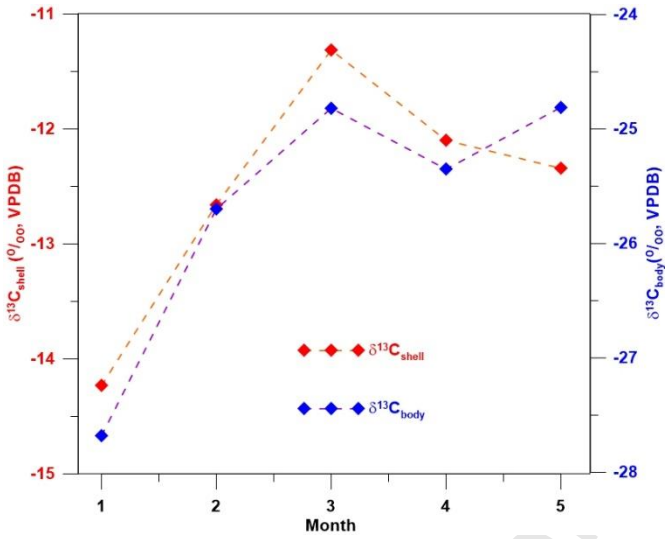

Figure 5. $\delta^{13}C$ variations of the snail shell (red) and body tissue (blue) $\delta^{13}C$ vs months

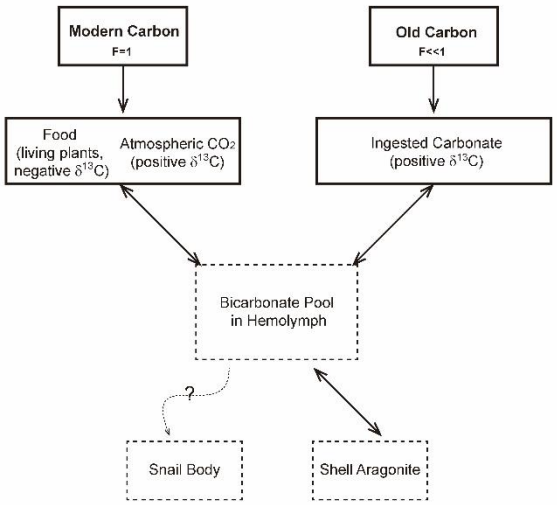

Figure 6. Simple framework showing potential carbon sources in snails.

Table 1. Stable isotopic and carbon-14 dating results from cultured snail tissues and shells and the calculated proportions of carbon from the snail diet, atmospheric CO_2 and carbonates.

Lab Code	Sample ID	Month	$F^{14}C$	±1σ	Apparent ^{14}C age (yrs)	± 1 σ	Shell weight (g)	$\delta^{13}C$ (‰)	Diet (%)	Atmosphere CO_2(%)	Carbonate (%)
XA17093	T28-1-5-S	1	0.8149	0.0023	1645	20	0.12	-14.23	69	12	19
XA17092	T28-2-5-S	2	0.7981	0.0020	1810	20	1.16	-12.66	59	21	20
XA17091	T28-3-5-S	3	0.7304	0.0020	2520	20	4.61	-11.31	44	29	27
XA17090	T28-4-5-S	4	0.7471	0.0019	2340	20	8.53	-12.10	50	25	25
XA17089	T28-5-5-S	5	0.8313	0.0022	1480	20	8.99	-12.34	61	22	17
XA14113	Snail egg shell		0.9124	0.0030	740	30		-14.17	80	11	9
XA13646	T28-1-5-B	1	0.9948	0.0030				-27.68	94	5	1
XA13645	T28-2-5-B	2	1.0042	0.0028				-25.70	86	15	0
XA13644	T28-3-5-B	3	1.0054	0.0031				-24.82	81	19	0
XA13643	T28-4-5-B	4	0.9995	0.0029				-25.35	83	17	0
XA13642	T28-5-5-B	5	1.0009	0.0028				-24.81	81	19	0

In the sample ID column, B: snail body, and S: snail shell

Table 2. Stable isotopic and carbon-14 dating results from the shells of wild snails

Lab Code	Location	Latitude (°N）	Longitude (°E)	$F^{14}C$	±1σ	^{14}C age (yr BP)	±1σ
XA20582	Luohe	33.62	114.07	0.8939	0.0034	900	30
XA20578	Fuyang	32.74	116.41	0.9319	0.0037	570	30
XA20588	Hefei	31.70	117.28	0.9840	0.0034	130	30
XA20587	Nanchang	28.80	115.87	0.9546	0.0033	370	30
XA20586	Ji‘an	27.08	114.99	0.9638	0.0034	300	30
XA20579	Shilin	24.86	103.43	0.9634	0.0034	300	30
XA16710	Xiamen	24.52	118.09	1.0107	0.0035	Modern	30
XA16517	Guangzhou	23.22	113.23	1.0208	0.0029	Modern	20
XA16709	Sanya	18.34	109.48	0.9966	0.0031	30	25